Topsmelt *Atherinops affinis* 7-Day Chronic Toxicity Test

1.0 OBJECTIVE

In laboratory tests designed to determine the toxicity of water samples, larval fish are exposed to test solutions for 7 days, after which the percentage mortality is determined in each toxicant concentration, and growth is measured by dry weight of larvae. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as dissolved oxygen, pH, salinity, ammonia, and temperature.

In this procedure, water samples collected from field stations are divided into replicate beakers in the laboratory. Five randomly selected larval fish are placed into each replicate container. Each beaker is monitored daily for mortality. After a 7-day exposure, survival is counted and recorded to give an estimate of sample toxicity. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (US EPA 1995).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (MPSL SOP 1.3).

2.1 Culture and Acclimation

- larval fish are obtained from Aquatic Bio Systems (Fort Collins, CO)
- airstones and portable aeration (pumps or compressed air or oxygen)
- 20-µm-filtered and 1-µm-filtered seawater from a uniform source
- brine shrimp *Artemia* cysts for producing *Artemia* nauplii
- disposable plastic pipets

2.2 Test Initiation

- 1000 mL clean glass beakers (5 per sample concentration)
- 1000 mL plastic tri-pour reference toxicant test containers
- 1000 mL volumetric flasks and pipettes for reference toxicant dilutions
- Plastic screen tube 150-µm mesh, 25 cm diameter for fish
- Plastic screen tube 100-µm mesh for *Artemia*
- Water bath or environmental chamber
- Randomization sheet to arrange and identify test containers
- Data sheets

• Gloves and appropriate safety gear (see MPSL lab safety manual)
• Sample vials for reference toxicant analysis (new polyethylene 60 mL)
• Graduated pipettes
• Analytical balance
• Plastic squirt bottles

2.3 Water Quality

• Meters and probes for measuring dissolved oxygen, pH, salinity, and ammonia
• Thermometers (glass mercury thermometer and continuously recording thermometer)
• Graduated pipettes and hand pipette pump for water quality sampling
• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

Dilution water consists of ambient Granite Canyon seawater, filtered to 1 µm, at ambient salinity (34 ± 2‰).

3.0 EXPERIMENTAL DESIGN

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of five replicate test beakers for each sample concentration. Beakers are arranged randomly, and each receives five randomly selected larval topsmelt. The quality of test fish and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Dissolved oxygen, pH, salinity, and ammonia are measured at the beginning of the exposure. New and old dissolved oxygen and pH are measured daily. Temperature is measured daily by hand and measured continuously by a temperature logger. The photoperiod for the test is 16 hours light: 8 hours dark, and the temperature is 20 ± 1°C.

4.0 SAMPLE PREPARATION

Label one-liter beakers as indicated on the randomization sheet generated for the test. Determine the salinity of the test solutions. Topsmelt may be tested at salinities of 5 - 45‰ as long as adequate acclimation of organisms is followed. Fish may not be subjected to more than 3‰ change per 24 hour period. Be sure to stir all samples before measuring salinity, and measure salinity immediately after stirring. There should be no more than a 2‰ difference between the highest salinity sample and the lowest. For testing at 34‰, sample salinities below 32‰ are adjusted

to 34‰ using hypersaline brine made from frozen seawater or artificial salts. Check the pH of brine. If necessary, adjust the brine pH by adding acid or sodium hydroxide until it is between 7.5-8.5.

Using the random number sheet, aliquot 200 mL of sample to beakers. Minimize sample exposure to sunlight (never leave samples in direct sunlight), and schedule loading times to avoid prolonged sample exposure to temperatures above 20°C.

5.0 CONTROLS

5.1 Seawater and Brine Controls

The seawater control consists of 1-µm filtered Granite Canyon seawater. If a brine control is necessary, it should be prepared to contain the same proportion of brine as the lowest salinity sample that was adjusted. See Salinity Adjustment Worksheet for calculations.

5.2 Reference Toxicant Test

A reference toxicant test must be conducted concurrently with every test to indicate the sensitivity of the organisms and the suitability of the test methodology. Copper chloride ($CuCl_2$) should be used as the reference toxicant for topsmelt tests, unless another toxicant is specified by the Regional Water Quality Control Board or other appropriate regulatory agency. Prepare a 10,000 µg/L copper stock solution by adding 0.0268g $CuCl_2$ to one liter of distilled water in a volumetric flask. Cap tightly and mix thoroughly. Sample the reference toxicant stock solution at the beginning of the test for chemical verification of the copper concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N reagent grade nitric acid. Reference toxicant solutions consist of five replicates of the following concentrations: 0, 56, 100, 180, and 320 µg/L. Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare 1000 mL of each concentration, and aliquot 200 mL to each of the five replicates.

All tests (sample and reference toxicant) must use fish from the same source and hatch. Larvae are 9 - 15 days post hatch at test initiation. They must be handled in the same way and delivered to the test containers at the same time.

6.0 TEST INITIATION

6.1 Water Quality Measurements

Before test initiation, measure dissolved oxygen, pH, salinity, and temperature on each concentration of the test and the reference toxicant test. Dissolved oxygen concentration should be 4.0 mg/L. If not, aerate gently until DO increased above 4.0 mg/L.

6.2 Randomized Loading of Topsmelt

Transfer larval topsmelt from their holding tub into test containers using disposable plastic pipettes or a fire-polished glass tube. Repeat this process until all beakers are loaded. Place acrylic covers over each set of beakers on a shelf. Maintain water temperature (20 ± 1°C) by sorting animals in the constant temperature room where the test is being held.

7.0 MONITORING THE TOXICITY TEST

7.1 Counting Topsmelt Mortality and Feeding

Test duration is 7 days. Check all test containers daily, and record the number of dead topsmelt. Immobile larvae that do not respond to a stimulus are considered dead. The stimulus should be a gentle stream of water from a Pasteur pipette. Larvae that exhibit any response clearly visible to the naked eye are considered living. Remove dead animals, *Artemia* and other debris. Containers are fed 40 freshly hatched *Artemia* nauplii per larvae twice daily.

7.2 Test Solution Renewal

Because toxicity may change over short time periods in test containers, the test solutions must be renewed daily. Dissolved oxygen concentrations should be checked on new water used and these samples must be aerated if oxygen concentrations exceed maximum values, or fall below minimum values allowed.

Remove 75% of the original test solution from each container, taking care to avoid losing or damaging topsmelt. This can be accomplished by siphoning with a small-bore (2 to 3 mm) fire-polished glass tube or pipette attached to non-toxic plastic tubing. It is useful to siphon into a beaker to catch wayward topsmelt so they can be returned to test containers undamaged. Siphon tubes must be leached for 24 hours prior to renewal in seawater.

To minimize disturbance to the topsmelt, refill the containers to the 200 ml mark by carefully pouring new test solution into the test containers using small diameter plastic tubing attached to a bent clean glass rod that directs incoming solution upward or to the side to slow the current and minimize turbulence. Feed 40 *Artemia* nauplii per larvae to each test container after the renewal solution has been added.

8.0 TERMINATING THE TOXICITY TEST

After 7 days of exposure final mortality counts are made. Final water quality (pH, temperature, dissolved oxygen, salinity) must be sampled at the termination of the test. Test containers are poured through a screen tube and fish caught on screen rinsed in Nanopure to remove salts and particulate matter. Fish are then tweezed off the screen and into pre-weighed pans. There is a separate pan for each test container. Place weigh pans in a 60°C oven for 24 hours. Allow pans to cool for one hour before weighing to the nearest 0.01 mg.

9.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and brine controls have acceptable survival. This toxicity test procedure is considered acceptable if larvae survival in the dilution control is ≥80%. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures range from 20 ± 1°C. Acceptable dissolved oxygen concentration is 60-100% saturation.

10.0 REFERENCES

U.S. Environmental Protection Agency. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. Office of Research and Development. EPA/600/R-95/136. August 1995

11.0 TEST SUMMARY

Species:	*Atherinops affinis*
Test Duration:	7 Days
Renewals:	Daily
Organism Source	Aquatic Bio Systems
Age of test organisms:	9 - 15 days post-hatch
Salinity:	Selection ± 2‰ recommended
Dissolved Oxygen	>4 mg/L recommended
Temperature:	20 ± 1°C recommended
Light intensity:	Ambient laboratory illumination 10-20 $\mu E/\mu^2/s$
Photoperiod:	16 hour Light: 8 hour Dark
Replication:	5
Test Containers:	1000 mL beakers
Test solution volume:	200 mL minimum
Loading:	5 animals per beaker
Feeding:	Feed 40 newly hatched Artemia nauplii per larvae twice daily
Water Quality:	Dissolved oxygen, pH, salinity, ammonia, and temperature
Reference Toxicant:	Copper chloride ($CuCl_2$)
Daily Monitoring:	Count number alive and remove dead
Acceptability Criteria:	Seawater and Brine Controls: ≥80%